Feature Review

CellPress

Special Issue: Circuit Development and Remodeling

Control of neural circuit formation by leucine-rich repeat proteins

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The function of neural circuits depends on the precise connectivity between populations of neurons. Increasing evidence indicates that disruptions in excitatory or inhibitory synapse formation or function lead to excitation/inhibition (E/I) imbalances and contribute to neurodevelopmental and psychiatric disorders. Leucine-rich repeat (LRR)-containing surface proteins have emerged as key organizers of excitatory and inhibitory synapses. Distinct LRR proteins are expressed in different cell types and interact with key pre- and postsynaptic proteins. These protein interaction networks allow LRR proteins to coordinate pre- and postsynaptic elements during synapse formation and differentiation, pathway-specific synapse development, and synaptic plasticity. LRR proteins, therefore, play a critical role in organizing synaptic connections into functional neural circuits, and their dysfunction may contribute to neuropsychiatric disorders.

LRR proteins and the organization of functional neural circuits

The function of neural circuits depends on the precise connectivity between populations of neurons. In the central nervous system (CNS) this is mediated by glutamatergic and GABAergic synapses, and there is emerging evidence that disruptions in the formation or function of excitatory or inhibitory synapses lead to excitation/inhibition (E/I) imbalances, which characterize several psychiatric and neurodevelopmental disorders [1–8]. These considerations underscore the importance of understanding the molecular control of excitatory and inhibitory synapse formation, and the signals that allow cell type-specific control of E/I balance.

In this review, we examine recent evidence indicating that cell surface proteins containing an extracellular LRR domain [9] are key organizers of excitatory and inhibitory synapses in the CNS. Distinct LRR proteins are expressed in different cell types, and are generally localized to the postsynaptic membrane, from where they exert a strong influence on the development of synaptic connections. LRR proteins interact with key components of the postsynaptic machinery and trans-synaptically couple to essential presynaptic receptors, which places them in an ideal position to coordinate pre- and postsynaptic differentiation during circuit formation. New insights indicate that the function of LRR proteins extends beyond the initial formation of synaptic contacts and point to a role in regulating functional properties and activity-dependent plasticity of synapses. These findings indicate that LRR proteins play a critical role in the organization and function of neural circuits.

LRR proteins as regulators of synapse development

Recent studies have identified several closely related LRR protein families as regulators of synapse development. A simple *in vitro* assay, which tests the ability of neurons to form synapses onto co-cultured heterologous cells expressing candidate genes [10,11], has been instrumental in identifying synapse-organizing or synaptogenic LRR proteins. The elucidation of their trans-synaptic interactions has highlighted a common theme of diverse postsynaptic ligands coupling to a limited repertoire of presynaptic receptors. In this review, we provide an inventory of these LRR proteins and discuss their roles in synapse development and function, focusing on the vertebrate system.

LRR transmembrane neuronal proteins control excitatory synapse development via distinct presynaptic partners

Since their original identification as synaptogenic proteins in a co-culture assay-based expression screen [12], LRR transmembrane neuronal proteins (LRRTMs) 1–4 have rapidly become the most intensively studied family of LRR-containing synaptic organizers. LRRTMs are type I transmembrane proteins with an extracellular LRR domain and a C-terminal postsynaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1) (PDZ) interaction site (Figure 1A), and distinct, partially overlapping expression patterns in the brain [13] (Figure 4). LRRTM2, the best characterized family member, localizes to the postsynaptic density of excitatory synapses and regulates postsynaptic differentiation by recruiting key elements of the synaptic machinery, including the scaffolding protein PSD-95 and glutamate receptor subunits [12,14]. LRRTM2, expressed on the surface of non-neuronal cells, induces presynaptic differentiation in contacting axons of co-cultured neurons, a property shared with LRRTM1, LRRTM4, and to a lesser degree, LRRTM3 [12,14,15].

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Keywords: synaptogenesis; synaptic adhesion; excitation/inhibition balance; connectivity; synaptic transmission; glutamate receptor.

0166-2236/
 http://dx.doi.org/10.1016/j.tins.2014.07.004

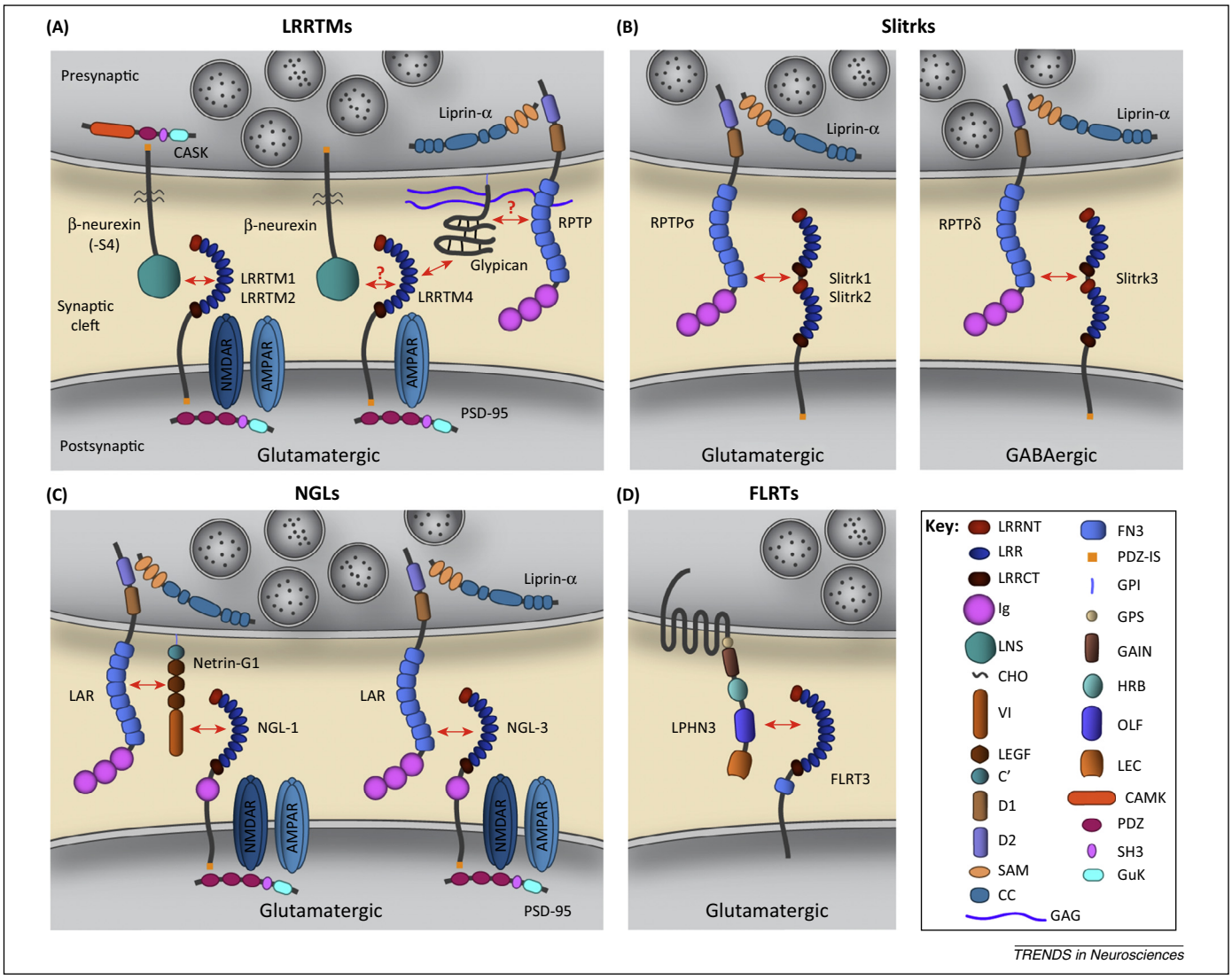

Figure 1. Synaptic leucine-rich repeat (LRR) proteins and their interactions[a]. Postsynaptic LRR proteins and their presynaptic partners form a trans-synaptic complex that bridges the synaptic cleft and recruits essential scaffolding molecules and neurotransmitter receptors to the synapse. **(A)** Regulation of excitatory synapse development by LRR transmembrane neuronal proteins (LRRTMs). LRRTM1 and LRRTM2 bind to presynaptic α- (long) and β- (short) neurexins that lack a small insert at splice site 4 (S4) in the LNS domain. Only β-neurexin is shown. LRRTM4 binds to presynaptic heparan sulfate proteoglycans (HSPGs), including glypican. Glypican may act via a co-receptor such as leukocyte common antigen-related (LAR) family receptor protein tyrosine phophatase (RPTPs) to induce presynaptic differentiation. LRRTM4 can also bind neurexin under certain conditions, but the functional significance of this interaction is not clear. The cytoplasmic tails of LRRTMs, neurexins and RPTPs couple to the scaffolding proteins PSD-95, CASK and liprin-α, respectively. **(B)** Slitrk1 and Slitrk2 bind to presynaptic RPTPσ and regulate excitatory synapse formation in cultured neurons. Slitrk3 binds to presynaptic RPTPδ and regulates inhibitory synapse formation. **(C)** NGL-1 binds to presynaptic Netrin-G1, and this interaction induces recruitment of LAR to the complex. NGL-2 binds to presynaptic Netrin-G2 (not shown). NGL-3 binds to the first two FN3 repeats in LAR. **(D)** FLRT3 binds to the adhesion GPCR latrophilin. [a]Domain abbreviations: LRRNT, LRRCT, LRR N- and C-terminal flanking domains; Ig, immunoglobulin-like; LNS, laminin-α/neurexin/sex-hormone-binding globulin (also known as laminin-G domain); CHO, carbohydrate attachment; VI, laminin N-terminal; LEGF, laminin EGF-like motifs 1-3; C′, C-terminal domain; D1, D2, membrane-proximal (catalytically active) and -distal (inactive) tyrosine phophatase domains; SAM, sterile alpha motif; CC, coiled coil; GAG, glycosaminoglycan (heparan sulfate); FN3, fibronectin type III; PDZ-IS, postsynaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1) (PDZ) interaction site; GPI, glycosylphosphatidylinositol; GPS, GPCR proteolytic site; GAIN, GPCR autoproteolysis-inducing domain; HRB, hormone-binding domain; OLF, olfactomedin; LEC, lectin domain; CaMK, Ca^{2+}/calmodulin-dependent kinase; SH3, *Src* homology 3; GuK, guanylate kinase domain.

LRRTM1 and LRRTM2 induce presynaptic differentiation by trans-synaptically binding to neurexins [14–16], a family of alternatively spliced receptors that organize presynaptic development and function [17,18]. Neurexins interact with a multitude of postsynaptic ligands, including the neuroligins and the cerebellin-glutamate receptor δ complex [19–21]. LRRTM binding to neurexin is regulated by alternative splicing: LRRTM1 and -2 only bind to neurexins lacking a small insert at splice site 4 (S4) [15,16]. Surprisingly, LRRTM4 uses a different mechanism to induce presynaptic differentiation. LRRTM4 binds to heparan sulfate proteoglycans (HSPGs), most prominently glypicans, and requires heparan sulfate (HS) on the presynaptic cell surface to induce synapse formation [22,23] (Figure 1A). Unlike neurexins, glycosylphosphatidylinositol (GPI)-anchored glypicans lack a cytoplasmic domain, raising the possibility of a transmembrane co-receptor to trigger presynaptic differentiation. The leukocyte common antigen-related (LAR) receptor protein tyrosine phosphatase binds glypican in *Drosophila* [24], and is a candidate for such a co-receptor.

Overexpression of LRRTM2 or LRRTM4 in cultured hippocampal neurons increases the density of excitatory, but not inhibitory, synapses [14,15,22,23]. LRRTMs most